

Chapter 2

Molecular Biology of Clear Cell Renal Carcinoma

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Kidney cancer is one of the ten most common cancers in the developed world [1]. Several histological variants of kidney cancer are recognized by pathologists including clear cell renal carcinoma, papillary (chromophil) renal carcinoma, chromophobic renal carcinoma, and oncocytoma [2]. The identification and study of rare families that are predisposed to kidney cancer led to the identifications of genes that, when mutated in the germline, confer an increased risk of the different histological variants of kidney cancer [3, 4]. For example, germline *VHL* mutations are linked to an increased risk of clear cell renal carcinoma, which is the most common form of kidney cancer. This chapter describes the molecular biology of clear cell renal carcinoma with an emphasis on the role of *VHL* in disease pathogenesis.

2.1 *VHL* Tumor Suppressor Gene: Role in *VHL* Disease and Kidney Cancer

Von Hippel-Lindau disease was first described over 100 years ago and is characterized by an increased risk of clear cell renal carcinoma, hemangioblastomas of the retina, cerebellum, and spinal cord, and intra-adrenal paragangliomas (also called pheochromocytomas) [5, 6]. Individuals with *VHL* disease have typically inherited a defective allele for the *VHL* tumor suppressor gene, located at chromosome 3p25, from one of their parents. Less commonly *VHL* disease is the result of a de novo *VHL* mutation leading to germline *VHL* mosaicism [7–9]. The development of overt pathology in *VHL* disease is linked to somatic inactivation or loss of the remaining wild-type *VHL* allele in a susceptible cell type.

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In keeping with this knowledge, biallelic *VHL* inactivation due to mutations or, less commonly, gene methylation, is also very common in sporadic (nonhereditary) clear cell renal carcinoma [10]. In most studies, at least 50% of such tumors have sustained *VHL* mutations, usually accompanied by macrodeletions affecting the remaining chromosome 3p arm. More recent studies that have employed more sensitive sequencing technologies suggest that 50% might significantly underestimate the frequency of *VHL* mutations in this population [11, 12].

Notably, the *VHL* gene is ubiquitously expressed, and yet *VHL* mutations are tightly linked to the development of kidney cancer but are rare in other common epithelial neoplasms. Why some cell types are susceptible to transformation after *VHL* inactivation and others are not is largely a mystery. The same, however, can be said of most cancer predisposition genes.

2.2 Cooperating Genetic Events in Kidney Cancer

Although *VHL* inactivation plays a critical role in hereditary (VHL disease-associated) and sporadic clear cell renal carcinoma, it is not sufficient to cause this disease. This has been most convincingly demonstrated by careful natural history studies of VHL patients. The kidneys of VHL patients frequently harbor hundreds of preneoplastic renal cysts, only a few of which will become malignant renal carcinomas [13, 14]. The development of preneoplastic renal cysts in this setting reflects the stochastic loss of the remaining wild-type *VHL* allele, suggesting that biallelic *VHL* inactivation is sufficient to cause cysts but not clear cell renal carcinoma. The latter presumably requires the accumulation of additional mutations affecting genes other than the *VHL* gene itself. In this regard, a number of recurrent chromosomal changes have been documented in hereditary and sporadic clear cell renal carcinoma including loss of chromosome 14q and gain of chromosome 5q [15–19]. In addition, cancer genome sequencing projects have revealed potentially pathogenic mutations of the known tumor suppressor genes *CDKN2A*, *TP53*, *NF2*, and *PTEN* in a subset of clear cell renal carcinomas [20]. These efforts have also revealed that a subset clear cell renal carcinoma have mutations affecting chromatin modifying genes such as *PRBM1*, *KDM6A*, and *SETD2* [20, 21]. The requirement for multiple, cooperating, genetic events to cause clear cell renal carcinoma (as appears to be true for most epithelial neoplasms) presumably explains why VHL patients typically develop kidney cancer in adulthood rather than as children.

2.3 Functions of the VHL Protein

The *VHL* gene encodes for two proteins by virtue of two alternative, in-frame, translation initiation sites [22–24]. The long form contains 213 amino acid residues. The short form lacks the first 53 amino acid residues of the long form. In most biochemical

and biological assays, the two isoforms behave similarly, and the mutations that have been detected in kidney cancer would affect both isoforms. Therefore, “pVHL” will be used to refer to both isoforms in this chapter for simplicity.

pVHL is primarily a cytosolic protein but dynamically shuttles back and forth between the cytosolic compartment and the nucleus [25–29]. Some pVHL can also be detected in association with the endoplasmic reticulum [30] and with mitochondria [31]. pVHL has a number of functions including roles in regulating protein turnover, primary cilium maintenance, microtubule stability, and extracellular matrix formation (reviewed in [32]). For example, loss of pVHL leads to loss of the primary cilium, a specialized structure on the cell surface that plays a role in signal transduction, including mechanical signals arising from fluid pressure and flow [33–36]. Interestingly, a number of genes that, like *VHL*, play roles in primary cilium maintenance have been linked, when altered, to the development of visceral cysts [37]. Therefore, loss of the primary cilium might contribute to the development of the visceral cysts that are a hallmark of VHL disease. Nonetheless, some VHL mutations that have been linked to human kidney cancer do not compromise pVHL’s role in primary cilium maintenance [35]. Instead, the pVHL function that appears to be most tightly linked to suppression of kidney cancer relates to its role in the regulation of the HIF transcription factor, as described below.

2.4 pVHL and HIF

pVHL is part of a multiprotein complex that contains elongin B, elongin C, Cul2, and Rbx1 (reviewed in [32]) (Fig. 2.1). This complex serves as a ubiquitin ligase complex, meaning that it can direct the polyubiquitylation of specific substrates, which then undergo proteasomal degradation. The best documented target of the pVHL ubiquitin complex is the heterodimeric transcription factor HIF (hypoxia-inducible factor), which is a master regulator of genes that promote the adaptation to hypoxia (low oxygen) such as genes that promote glycolysis as an alternative ATP source, genes that promote erythropoiesis (such as erythropoietin), and genes that promote angiogenesis (such as VEGF).

HIF consists of an unstable alpha subunit and a stable beta subunit. In the presence of oxygen, the alpha subunit becomes hydroxylated on one (or both) of two specific prolyl residues [38–42]. Hydroxylation of either site generates a pVHL docking site and sets in motion the polyubiquitylation and destruction of HIF α . When oxygen levels are low, or pVHL is defective, HIF α is not polyubiquitylated and instead accumulates, dimerizes with HIF β , and transcriptionally activates HIF target genes such as VEGF. Accordingly, deregulation of HIF target genes is a signature of pVHL-defective kidney cancers [43–46]. Overproduction of VEGF and erythropoietin can explain the clinical observations that kidney cancers are highly angiogenic and capable of inducing paraneoplastic erythrocytosis, respectively.

There are three HIF α genes in the human genome (HIF1 α , HIF2 α , and HIF3 α). The products of all three of these genes have the ability to heterodimerize with HIF β

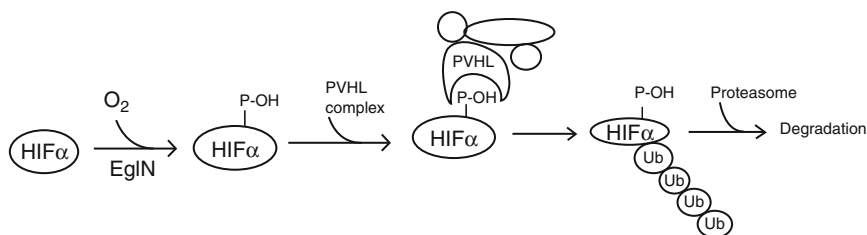


Fig. 2.1 Regulation of HIF by pVHL. In the presence of oxygen, HIF α family members are hydroxylated on one (or both) of two conserved prolyl residues (one is shown for simplicity) by members of the EglN family. This generates a binding site for pVHL, which recruits a ubiquitin-conjugating enzyme (the other components of the pVHL ubiquitin ligase are not labeled for simplicity). Once polyubiquitylated, HIF α undergoes proteasomal degradation

family members. The resulting DNA-binding complexes formed by HIF1 α and HIF2 α can activate transcription. HIF1 α is widely expressed while the expression of HIF2 α is restricted to certain cells and tissues. In addition, it is clear that some HIF-responsive genes, such as many glycolytic genes [47], are primarily regulated by HIF1 α , while others, such as erythropoietin and the stem cell factor Oct4, are primarily under the control of HIF2 α [48, 49]. HIF3 α has been less intensively studied than HIF1 α and HIF2 α . It appears to undergo extensive mRNA splicing, with some mRNA isoforms encoding proteins that can block the action of HIF1 α and HIF2 α [50–54].

2.5 HIF and Kidney Cancer

Deregulation of HIF2 α appears to be a driving force in pVHL-defective clear cell renal carcinomas. In preclinical models, restoring the function of pVHL in pVHL-defective renal carcinoma lines suppresses their ability to form tumors in immunocompromised mice [25, 45]. Importantly, this activity of pVHL can be overridden by restoring HIF2 α activity, suggesting that downregulation of HIF2 α is necessary for tumor suppression by pVHL [55, 56]. Moreover, eliminating HIF2 α in pVHL-defective renal carcinoma lines inhibits tumor growth, indicating that downregulation of HIF2 α is also sufficient for tumor suppression by pVHL [57, 58]. Deregulation of HIF2 α also appears to be necessary and sufficient for much of the pathology observed in mice that have been engineered to lack pVHL in specific tissues [59, 60]. The risk of kidney cancer associated with different *VHL* alleles correlates with the degree to which those alleles deregulate HIF2 α (as well as HIF1 α) [61] and germline HIF2 α polymorphisms have been linked to the risk of sporadic kidney cancer [62].

In stark contrast, HIF1 α exhibits properties of a tumor suppressor in clear cell renal carcinoma (Fig. 2.2). Although increased HIF2 α levels are a hallmark of clear cell renal carcinoma cell lines and tumors, many clear cell renal carcinoma lines and

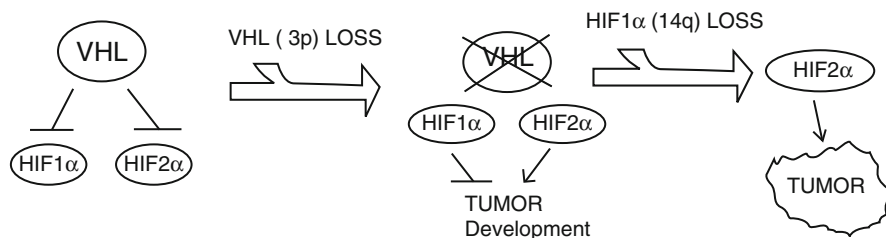


Fig. 2.2 Roles of HIF paralogs in kidney cancer. Loss of pVHL leads to the accumulation of both HIF1 α and HIF2 α . HIF1 α has tumor suppressor properties, and HIF2 α has oncogenic properties. Diminished HIF1 α activity, such as through loss of chromosome 14q, contributes to tumor development. Tumors that retain HIF1 α activity presumably harbor mutations at other genetic loci that inure them to the tumor suppressor activity of HIF1 α .

tumors produce low or undetectable levels of HIF1 α [44, 63, 64]. *HIF1 α* is located on chromosome 14q, which is deleted in ~40% of clear cell renal carcinomas, and 14q-deleted tumors exhibit a gene expression signature indicative of HIF1 α loss [64]. In the majority of such tumors the remaining wild-type *HIF1 α* allele appears to be wild type, suggesting that loss of one copy (haploinsufficiency) of *HIF1 α* can have a biological effect. In contrast to tumors, a significant proportion of pVHL-defective renal carcinoma lines have sustained focal, homozygous, deletions, rendering them null for wild-type *HIF1 α* [64]. This suggests that reduction to nullizygosity is a late event in tumor progression (most cell lines are established from late stage disease) and/or is selected for during the establishment and propagation of such cell lines.

Eliminating HIF1 α in pVHL-defective renal carcinoma lines that express both HIF1 α and HIF2 α enhances their ability to proliferate in vitro and in vivo, while restoring HIF1 α levels in pVHL-defective renal carcinoma lines lacking HIF1 α has the opposite effect [64]. Although rare, intragenic HIF1 α mutations have been described in human clear cell tumors, and when tested, these mutations impair HIF1 α 's ability to suppress proliferation in such assays [20, 64, 65]. Collectively, these results indicate that HIF1 α has the credentials of a tumor suppressor.

The mechanistic basis for the opposing effects of HIF1 α and HIF2 α is not completely understood. As discussed above, the sets of genes regulated by HIF1 α and HIF2 α overlap but are not entirely congruent. Perhaps one or more genes that are preferentially regulated by HIF1 α can suppress renal carcinoma growth. In addition, HIF1 α , and to a much lesser extent HIF2 α , is subject to a second layer of oxygen-dependent regulation. Specifically, in the presence of oxygen, HIF1 α becomes hydroxylated on an asparaginyl residue by the enzyme FIH-1, which diminishes the ability of HIF1 α to activate transcription [66–68]. HIF2 α is relatively insensitive to FIH-1 [69, 70]. Accordingly, displacement of HIF2 α from HIF target genes by HIF1 α in an oxygenated, pVHL-defective tumor might lead to a decrease in transcription because the former is more active than the latter as a transcriptional activator. Finally, the different effects of HIF1 α and HIF2 α with respect to renal suppression might relate to differential interactions with other transcription factors such as c-Myc [63, 71] and Notch [72].

In addition to HIF, pVHL regulates, at least indirectly, the oncogenic transcription factors NF κ B and β -catenin. pVHL serves as an adaptor protein to promote the inhibitory phosphorylation of the NF κ B agonist Card9, leading to decreased NF κ B activity [73]. In addition, there is evidence for cross talk between HIF and NF κ B, HIF inducing NF κ B activity in some systems and NF κ B, in turn, promoting the transcription of HIF1 α [74–76]. Deregulation of NF κ B might contribute to the resistance of clear cell renal carcinomas to cytotoxic agents. In addition, some pathogenically relevant transcriptional targets of HIF, such as VEGF and cyclin D1, are also NF κ B targets, suggesting that HIF and NF κ B conspire to promote tumorigenesis.

pVHL also stabilizes the putative tumor suppressor Jade1, which has been implicated in polyubiquitylation of β -catenin [77, 78]. In addition, loss of pVHL leads to increased signaling downstream of receptors such as c-Met, leading to enhanced β -catenin activity [79]. Finally, there is evidence that HIF can, directly or indirectly, increase the transcription of β -catenin target genes [80]. β -Catenin likely contributes to abnormal proliferation and enhanced invasiveness.

2.6 Therapeutic Targets

2.6.1 *HIF*

In general, DNA-binding transcription factors, with the exception of steroid hormone receptors, have historically been difficult to inhibit with drug-like small organic molecules. A number of drugs have been identified, however, that indirectly downregulate HIF activity [81–89]. A caveat, however, is that HIF α has a very high metabolic turnover rate and, accordingly, is one of the first proteins to disappear from cells when transcription or translation are impaired. It is not clear whether some of the purported HIF inhibitors are specific for HIF or would have similar effects on other short-lived proteins. A second caveat is that most of the reported HIF inhibitors have been studied primarily with respect to HIF1 α rather than HIF2 α . For the reasons outlined above, it will be important to inhibit HIF2 α in clear cell renal carcinoma.

2.6.2 *mTOR*

Rapamycin-like mTOR inhibitors can downregulate HIF [90–95] and have been approved for the treatment of kidney cancer in patients who have failed treatment with VEGF inhibitors (see below). The antitumor activity of rapalogs is likely to reflect direct effects on tumor cells [96, 97], including downregulation of HIF, as well as effects on signaling downstream of VEGF in endothelial cells. pVHL-defective renal carcinoma cells appear to be more sensitive to rapalogs than identical cells that

contain wild-type pVHL [97]. Two factors, however, might limit the effectiveness of rapalogs in clear cell renal carcinoma. First, mTOR exists in a rapamycin-sensitive complex called TORC1 and a relatively insensitive complex called TORC2 [98]. Loss of TORC1 activity primarily affects HIF1 α , while loss of TORC2 primarily affects HIF2 α [99]. For this reason, ATP-competitive mTOR inhibitors capable of inhibiting both TORC1 and TORC2 might be more active than rapalogs for the treatment of clear cell carcinoma. A recent preclinical study supports this view [100]. Secondly, inhibition of TORC1 disrupts a negative feedback loop that normally serves to suppress signaling by specific receptor tyrosine kinases [101–103]. As a result, use of rapalogs has been associated with a paradoxical increase in receptor tyrosine kinase signaling. This increase, at least in theory, could be blunted by dual inhibition of PI3K and/or TORC2.

2.6.3 VEGF

Increased angiogenesis is a hallmark of kidney cancer and is associated with massive overproduction of the canonical HIF target VEGF. Multiple agents that inhibit VEGF itself, such as bevacizumab [104], or its receptor KDR, including sunitinib [105], sorafenib [106], and pazopanib [107, 108], have demonstrated activity in the treatment of this disease. Approximately, 70% of kidney cancer patients treated with VEGF inhibitors will experience disease stabilization and some degree of tumor shrinkage. The percentage of patients achieving a partial response by RECIST criteria varies, however, among the different agents. This might reflect differences in potency or fortuitous off-target effects that contribute to tumor regression.

It is hoped that second generation VEGF inhibitors that display increased potency and/or specificity will ultimately prove even more efficacious and less toxic than the currently approved agents. A caveat, however, is that early clinical data suggest that on-target toxicities, such as endothelial and cardiac dysfunction, will ultimately limit the degree to which VEGF signaling can be safely disrupted in man [109–113].

VEGF inhibitors favorably change the natural history of kidney cancer but are not curative. Kidney cancers treated with VEGF inhibitors will eventually develop resistance, as would be expected when treating a genetically complex neoplasm with a single agent. The mechanisms underlying the development of resistance to VEGF inhibitors in this setting are poorly understood. One recent preclinical study suggested a role for increased expression of interleukin-8, which is an angiogenic factor that can cooperate with VEGF [114, 115]. Interestingly, a recent clinical study linked interleukin-8 polymorphisms to the therapeutic efficacy of VEGF inhibitors [116].

There are conflicting studies with respect to importance of VHL status as a predictive biomarker with respect to the use of VEGF inhibitors for kidney cancer [117–120]. This might reflect, at least partly, variability among these studies with respect to histological inclusion criteria, response criteria, and the methods used to

determine VHL status. Suffice it to say that VHL status should not currently be used to guide the use of VEGF inhibitors in kidney cancer, especially given the paucity of alternative treatments.

2.6.4 PDGF

In preclinical models newly sprouting blood vessels become less sensitive to VEGF blockade once they are properly invested with surrounding pericytes [121–123], which respond to the HIF-responsive growth factor PDGF B [124–126]. Many of the available KDR inhibitors also inhibit the PDGF receptor, and hence, they might be ideally suited for blocking HIF-induced angiogenesis. On the other hand, the PDGFR inhibitor imatinib has not demonstrated activity in the treatment of kidney cancer, either alone or combined with bevacizumab [127–129].

2.6.5 TIE2

The TIE2 tyrosine kinase influences the response of endothelial cells to VEGF. TIE2 is under the control of two ligands, called angiopoietin 1 and angiopoietin 2 [130]. The former, which acts as a TIE2 agonist, stabilizes blood vessels, while angiopoietin 2, which acts as an antagonist, destabilizes blood vessels and primes them for sprouting in response to signals such as VEGF. At the same time, loss of TIE2 activity renders cells hypersensitive to VEGF withdrawal. Although there are conflicting data with respect to the regulation of angiopoietins by pVHL [131, 132], these considerations suggest that TIE2 inhibition might augment the activity of VEGF antagonists.

2.6.6 HGF and c-MET

pVHL-defective clear cell renal carcinoma cells are hypersensitive to the c-Met ligand HGF, leading to enhanced proliferation and invasiveness [133]. pVHL might regulate c-Met itself, which has been suggested to be a HIF target gene, as well as signaling downstream of c-Met [134–136]. Interestingly, both HGF and c-Met are located on chromosome 7, which is often amplified in clear cell renal carcinoma, and pVHL-defective cells are more dependent on c-Met for survival than are isogenic cells in which pVHL function has been restored [137]. c-Met has also been implicated in tumor angiogenesis. All of these considerations suggest that c-Met blockade, alone or in conjunction with a VEGF antagonist, would have activity in clear cell kidney cancer. c-Met inhibitors are already being tested in papillary renal cancer because some hereditary papillary renal cancers are linked to activating

germline c-Met mutations [3]. On the other hand, c-Met mutations appear to be rare in sporadic papillary renal cancers.

2.6.7 *TGF α and EGFR*

Kidney cancers frequently overproduce the HIF-responsive growth factor TGF α and its receptor EGFR [138–147]. Moreover, inhibiting this growth factor or its receptor in preclinical models suppresses pVHL-defective tumor growth [147, 148]. Nonetheless, the activity of small molecule EGFR inhibitors in the treatment of human kidney cancer has so far been disappointing [129, 149–151], although one study suggested a possible benefit for those with the highest expression of EGFR [150]. It should be borne in mind, however, that ATP-competitive EGFR inhibitors, such as erlotinib, have so far only proven efficacious for human cancers that have EGFR point mutations (in contrast to tumors driven by receptor overexpression). It is possible that anti-EGFR antibodies will be more efficacious in this setting, such as appears to be the case in colorectal cancer (where EGFR mutations are rare). On the other hand, the clinical results with two such agents as monotherapy, ABX-EGF and C225 (cetuximab), have thus far also been disappointing [152, 153].

A possible explanation for the ineffectiveness of EGFR blockade in kidney cancer relates to c-Met. There is a growing appreciation that c-Met activation can confer resistance to EGFR inhibitors in other settings [154–156], and as described above, c-Met is likely active in clear cell renal carcinoma. In this regard, it is worth noting that mouse models can underestimate the importance of c-Met, because mouse HGF does not effectively engage the human c-Met present on implanted human tumor cells [157]. These considerations warrant testing EGFR inhibitory antibodies in conjunction with c-Met inhibitors for the treatment of clear cell renal carcinoma.

2.6.8 *Cyclin D1 and Cdk6*

In renal epithelial cells, but not in many other cell types, the increased HIF levels observed upon pVHL loss drives the overproduction of cyclin D1 [158, 159]. Cyclin D1, bound to either cdk4 or cdk6, drives cell proliferation by phosphorylating, and thereby inactivating, the RB tumor suppressor protein. Cdk6 is located on a region of chromosome 7 that is amplified in clear cell renal carcinoma, and pVHL-defective renal carcinoma cells display increased sensitivity to cdk6 loss compared to cells in which pVHL function has been restored [137]. Some clear cell renal carcinomas harbor chromosome 9p deletions that target the cdk4/6 inhibitor p16 (also called Ink4A) [18, 160]. Importantly, however, mutations of pRB itself are rare in clear cell carcinoma, suggesting that they would retain sensitivity to inhibition of cdk4 and cdk6 (these kinases appear to be dispensable for oncogenesis in cells that lack wild-type pRB).

2.6.9 IL-6

Interleukin-6 is frequently overexpressed in clear cell renal carcinoma and might contribute to the unexplained fevers observed in a subset of kidney cancer patients [161–165]. IL-6 has also been reported to be regulated by pVHL [158]. IL-6 can act as an autocrine factor to stimulate renal carcinoma proliferation through activation of the JAK-STAT pathway [166]. In one clinical study, disease stabilization was noted in a subset of kidney cancer patients treated with a neutralizing anti-IL-6 antibody [167].

2.6.10 *Lactate Dehydrogenase A and Monocarboxylate Transporters*

HIF both reduces oxidation phosphorylation and increases the rate of glycolysis. The conversion of pyruvate to lactate is catalyzed by the HIF-responsive gene product LDH A, and the maintenance of intracellular pH is accomplished, at least partly, by upregulation of the HIF-responsive monocarboxylate transporter MCT4 [168–170]. Preclinical studies, conducted largely in other tumor types, suggest that blocking LDH A or MCT4 in pVHL-defective tumors would have antitumor effects [171–174].

2.7 Summary

Inactivation of the VHL tumor suppressor gene is a signature lesion in clear cell renal carcinoma. pVHL has multiple functions including targeting HIF family members for polyubiquitylation and proteasomal degradation. Deregulation of HIF2 α is a driving force in pVHL-defective tumors. HIF1 α , in stark contrast, exhibits properties of a tumor suppressor and appears to be one of the targets of the chromosome 14q deletions that are common in this disease. Drugs that inhibit the HIF target VEGF are now approved for the treatment of kidney cancer as are two rapamycin-like drugs that inhibit mTOR. The activity of mTOR inhibitors is probably due, at least in part, to tumor cell-intrinsic effects as well as effects on angiogenesis. A number of other HIF targets, in addition to VEGF, are suspected of contributing to tumor growth and can now be explored in the clinic.

References

1. Jemal A, Siegel R, Xu J, Ward E (2010) Cancer statistics, 2010. *CA Cancer J Clin* 60(5):277–300
2. Lopez-Beltran A, Carrasco JC, Cheng L, Scarpelli M, Kirkali Z, Montironi R (2009) 2009 update on the classification of renal epithelial tumors in adults. *Int J Urol* 16(5):432–443

3. Linehan WM, Zbar B (2004) Focus on kidney cancer. *Cancer Cell* 6(3):223–228
4. Linehan WM, Srinivasan R, Schmidt LS (2010) The genetic basis of kidney cancer: a metabolic disease. *Nat Rev Urol* 7(5):277–285
5. Kaelin WG (2002) Molecular basis of the VHL hereditary cancer syndrome. *Nat Rev Cancer* 2(9):673–682
6. Kaelin WJ, Maher E (1998) The VHL tumour-suppressor gene paradigm. *Trends Genet* 14:423–426
7. Sgambati M, Stolle C, Choyke P, Walther M, Zbar B, Linehan W, Glenn G (2000) Mosaicism in von Hippel-Lindau disease: lessons from kindreds with germline mutations identified in offspring with mosaic parents. *Am J Hum Genet* 66:84–91
8. Murgia A, Martella M, Vinanzi C, Polli R, Perilongo G, Opocher G (2000) Somatic mosaicism in von Hippel-Lindau disease. *Hum Mutat* 15:114
9. Hes FJ, McKee S, Taphoorn MJ, Rehal P, van Der Luijt RB, McMahon R, van Der Smagt JJ, Dow D, Zewald RA, Whittaker J, Lips CJ, MacDonald F, Pearson PL, Maher ER (2000) Cryptic von Hippel-Lindau disease: germline mutations in patients with haemangioblastoma only. *J Med Genet* 37(12):939–943
10. Kim WY, Kaelin WG (2004) Role of VHL gene mutation in human cancer. *J Clin Oncol* 22:4991–5004
11. Nickerson ML, Jaeger E, Shi Y, Durocher JA, Mahurkar S, Zaridze D, Matveev V, Janout V, Kollarova H, Bencko V, Navratilova M, Szeszenia-Dabrowska N, Mates D, Mukeria A, Holcatova I, Schmidt LS, Toro JR, Karami S, Hung R, Gerard GF, Linehan WM, Merino M, Zbar B, Boffetta P, Brennan P, Rothman N, Chow WH, Waldman FM, Moore LE (2008) Improved identification of von Hippel-Lindau gene alterations in clear cell renal tumors. *Clin Cancer Res* 14(15):4726–4734
12. Young AC, Craven RA, Cohen D, Taylor C, Booth C, Harnden P, Cairns DA, Astuti D, Gregory W, Maher ER, Knowles MA, Joyce A, Selby PJ, Banks RE (2009) Analysis of VHL gene alterations and their relationship to clinical parameters in sporadic conventional renal cell carcinoma. *Clin Cancer Res* 15(24):7582–7592
13. Mandriota SJ, Turner KJ, Davies DR, Murray PG, Morgan NV, Sowter HM, Wykoff CC, Maher ER, Harris AL, Ratcliffe PJ, Maxwell PH (2002) HIF activation identifies early lesions in VHL kidneys: evidence for site-specific tumor suppressor function in the nephron. *Cancer Cell* 1(5):459–468
14. Montani M, Heinimann K, von Teichman A, Rudolph T, Perren A, Moch H (2010) VHL-gene deletion in single renal tubular epithelial cells and renal tubular cysts: further evidence for a cyst-dependent progression pathway of clear cell renal carcinoma in von Hippel-Lindau disease. *Am J Surg Pathol* 34(6):806–815
15. Pei J, Feder MM, Al-Saleem T, Liu Z, Liu A, Hudes GR, Uzzo RG, Testa JR (2010) Combined classical cytogenetics and microarray-based genomic copy number analysis reveal frequent 3;5 rearrangements in clear cell renal cell carcinoma. *Genes Chromosomes Cancer* 49(7):610–619
16. Klatte T, Rao PN, de Martino M, LaRochelle J, Shuch B, Zomorodian N, Said J, Kabbavar FF, Belldegrun AS, Pantuck AJ (2009) Cytogenetic profile predicts prognosis of patients with clear cell renal cell carcinoma. *J Clin Oncol* 27(5):746–753
17. Chen M, Ye Y, Yang H, Tamboli P, Matin S, Tannir NM, Wood CG, Gu J, Wu X (2009) Genome-wide profiling of chromosomal alterations in renal cell carcinoma using high-density single nucleotide polymorphism arrays. *Int J Cancer* 125(10):2342–2348
18. Beroukhi R, Brunet JP, Di Napoli A, Mertz KD, Seeley A, Pires MM, Linhart D, Worrell RA, Moch H, Rubin MA, Sellers WR, Meyerson M, Linehan WM, Kaelin WG Jr, Signoretti S (2009) Patterns of gene expression and copy-number alterations in von-Hippel Lindau disease-associated and sporadic clear cell carcinoma of the kidney. *Cancer Res* 69(11):4674–4681
19. Yoshimoto T, Matsuura K, Karnan S, Tagawa H, Nakada C, Tanigawa M, Tsukamoto Y, Uchida T, Kashima K, Akizuki S, Takeuchi I, Sato F, Mimata H, Seto M, Moriyama M (2007) High-resolution analysis of DNA copy number alterations and gene expression in renal clear cell carcinoma. *J Pathol* 213(4):392–401

20. Dalglish GL, Furge K, Greenman C, Chen L, Bignell G, Butler A, Davies H, Edkins S, Hardy C, Latimer C, Teague J, Andrews J, Barthorpe S, Beare D, Buck G, Campbell PJ, Forbes S, Jia M, Jones D, Knott H, Kok CY, Lau KW, Leroy C, Lin ML, McBride DJ, Maddison M, Maguire S, McLay K, Menzies A, Mironenko T, Mulderrig L, Mudie L, O'Meara S, Pleasance E, Rajasingham A, Shepherd R, Smith R, Stebbings L, Stephens P, Tang G, Tarpey PS, Turrell K, Dykema KJ, Khoo SK, Petillo D, Wondergem B, Anema J, Kahnoski RJ, Teh BT, Stratton MR, Futreal PA (2010) Systematic sequencing of renal carcinoma reveals inactivation of histone modifying genes. *Nature* 463(7279):360–363
21. Varela I, Tarpey P, Raine K, Huang D, Ong CK, Stephens P, Davies H, Jones D, Lin ML, Teague J, Bignell G, Butler A, Cho J, Dalglish GL, Galappaththige D, Greenman C, Hardy C, Jia M, Latimer C, Lau KW, Marshall J, McLaren S, Menzies A, Mudie L, Stebbings L, Largaespada DA, Wessels LF, Richard S, Kahnoski RJ, Anema J, Tuveson DA, Perez-Mancera PA, Mustonen V, Fischer A, Adams DJ, Rust A, Chan-on W, Subimerb C, Dykema K, Furge K, Campbell PJ, Teh BT, Stratton MR, Futreal PA (2011) Exome sequencing identifies frequent mutation of the SWI/SNF complex gene PBRM1 in renal carcinoma. *Nature* 469(7331):539–542
22. Schoenfeld A, Davidowitz E, Burk R (1998) A second major native von Hippel-Lindau gene product, initiated from an internal translation start site, functions as a tumor suppressor. *Proc Natl Acad Sci USA* 95(19):8817–8822
23. Blankenship C, Naglich J, Whaley J, Seizinger B, Kley N (1999) Alternate choice of initiation codon produces a biologically active product of the von Hippel Lindau gene with tumor suppressor activity. *Oncogene* 18:1529–1535
24. Iliopoulos O, Ohh M, Kaelin W (1998) pVHL19 is a biologically active product of the von Hippel-Lindau gene arising from internal translation initiation. *Proc Natl Acad Sci USA* 95:11661–11666
25. Iliopoulos O, Kibel A, Gray S, Kaelin WG (1995) Tumor suppression by the human von Hippel-Lindau gene product. *Nat Med* 1(8):822–826
26. Corless CL, Kibel A, Iliopoulos O, Kaelin WGJ (1997) Immunostaining of the von Hippel-Lindau gene product (pVHL) in normal and neoplastic human tissues. *Human Path* 28:459–464
27. Duan DR, Humphrey JS, Chen DYT, Weng Y, Sukegawa J, Lee S, Gnarr JR, Linehan WM, Klausner RD (1995) Characterization of the VHL tumor suppressor gene product: localization, complex formation, and the effect of natural inactivating mutations. *Proc Natl Acad Sci USA* 92:6495–6499
28. Lee S, Chen DYT, Humphrey JS, Gnarr JR, Linehan WM, Klausner RD (1996) Nuclear/cytoplasmic localization of the von Hippel-Lindau tumor suppressor gene product is determined by cell density. *Proc Natl Acad Sci* 93:1770–1775
29. Lee S, Neumann M, Stearman R, Stauber R, Pause A, Pavlakis G, Klausner R (1999) Transcription-dependent nuclear-cytoplasmic trafficking is required for the function of the von Hippel-Lindau tumor suppressor protein. *Mol Cell Biol* 19(2):1486–1497
30. Schoenfeld A, Davidowitz E, Burk R (2001) Endoplasmic reticulum/cytosolic localization of von Hippel-Lindau gene products is mediated by a 64-amino acid region. *Int J Cancer* 91:457–467
31. Shiao YH, Resau JH, Nagashima K, Anderson LM, Ramakrishna G (2000) The von Hippel-Lindau tumor suppressor targets to mitochondria. *Cancer Res* 60(11):2816–2819
32. Kaelin WG (2007) von Hippel-Lindau disease. *Annu Rev Pathol: Mech Dis* 2:145–173
33. Lutz MS, Burk RD (2006) Primary cilium formation requires von Hippel-Lindau gene function in renal-derived cells. *Cancer Res* 66(14):6903–6907
34. Esteban MA, Harten SK, Tran MG, Maxwell PH (2006) Formation of primary cilia in the renal epithelium is regulated by the von Hippel-Lindau tumor suppressor protein. *J Am Soc Nephrol* 17(7):1801–1806
35. Thoma CR, Frew IJ, Hoerner CR, Montani M, Moch H, Krek W (2007) pVHL and GSK3 β are components of a primary cilium-maintenance signalling network. *Nat Cell Biol* 9(5):588–595

36. Schraml P, Frew IJ, Thoma CR, Boysen G, Struckmann K, Krek W, Moch H (2009) Sporadic clear cell renal cell carcinoma but not the papillary type is characterized by severely reduced frequency of primary cilia. *Mod Pathol* 22(1):31–36
37. Zhang Q, Taulman PD, Yoder BK (2004) Cystic kidney diseases: all roads lead to the cilium. *Physiology (Bethesda)* 19:225–230
38. Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, Salic A, Asara J, Lane W, Kaelin WJ (2001) HIF α targeted for VHL-mediated destruction by proline hydroxylation: implications for O₂ sensing. *Science* 292:464–468
39. Jaakkola P, Mole D, Tian Y, Wilson M, Gielbert J, Gaskell S, Kriegsheim A, Hestreit H, Mukherji M, Schofield C, Maxwell P, Pugh C, Ratcliffe P (2001) Targeting of HIF- α to the von Hippel-Lindau ubiquitylation complex by O₂-regulated prolyl hydroxylation. *Science* 292:468–472
40. Masson N, Willam C, Maxwell P, Pugh C, Ratcliffe P (2001) Independent function of two destruction domains in hypoxia-inducible factor- α chains activated by prolyl hydroxylation. *EMBO* 20(18):5197–5206
41. Huang J, Zhao Q, Mooney SM, Lee FS (2002) Sequence determinants in hypoxia-inducible factor-1 α for hydroxylation by the prolyl hydroxylases PHD1, PHD2, and PHD3. *J Biol Chem* 277(42):39792–39800
42. Yu F, White S, Zhao Q, Lee F (2001) HIF-1 α binding to VHL is regulated by stimulus-sensitive proline hydroxylation. *Proc Natl Acad Sci USA* 98:9630–9635
43. Iliopoulos O, Jiang C, Levy AP, Kaelin WG, Goldberg MA (1996) Negative regulation of hypoxia-inducible genes by the von Hippel-Lindau protein. *Proc Natl Acad Sci* 93:10595–10599
44. Maxwell P, Weisner M, Chang G-W, Clifford S, Vaux E, Pugh C, Maher E, Ratcliffe P (1999) The von Hippel-Lindau gene product is necessary for oxygen-dependent proteolysis of hypoxia-inducible factor α subunits. *Nature* 399:271–275
45. Gnarra JR, Zhou S, Merrill MJ, Wagner J, Krumm A, Papavassiliou E, Oldfield EH, Klausner RD, Linehan WM (1996) Post-transcriptional regulation of vascular endothelial growth factor mRNA by the VHL tumor suppressor gene product. *Proc Natl Acad Sci* 93:10589–10594
46. Siemeister G, Weindel K, Mohrs K, Barleon B, Martiny-Baron G, Marme D (1996) Reversion of deregulated expression of vascular endothelial growth factor in human renal carcinoma cells by von Hippel-Lindau tumor suppressor protein. *Cancer Res* 56:2299–2301
47. Hu C-J, Wang L-Y, Chodosh LA, Keith B, Simon MC (2003) Differential roles of hypoxia-inducible factor 1 α (HIF-1 α) and HIF-2 α in hypoxic gene regulation. *Mol Cell Biol* 23(24):9361–9374
48. Covello KL, Kehler J, Yu H, Gordan JD, Arsham AM, Hu CJ, Labosky PA, Simon MC, Keith B (2006) HIF-2 α regulates Oct-4: effects of hypoxia on stem cell function, embryonic development, and tumor growth. *Genes Dev* 20(5):557–570
49. Rankin EB, Biju MP, Liu Q, Unger TL, Rha J, Johnson RS, Simon MC, Keith B, Haase VH (2007) Hypoxia-inducible factor-2 (HIF-2) regulates hepatic erythropoietin in vivo. *J Clin Invest* 117(4):1068–1077
50. Makino Y, Kanopka A, Wilson WJ, Tanaka H, Poellinger L (2002) Inhibitory PAS domain protein (IPAS) is a hypoxia-inducible splicing variant of the hypoxia-inducible factor-3 α locus. *J Biol Chem* 277(36):32405–32408
51. Makino Y, Uenishi R, Okamoto K, Ise T, Hosono O, Tanaka H, Kanopka A, Poellinger L, Haneda M, Morimoto C (2007) Transcriptional up-regulation of inhibitory PAS domain protein gene expression by hypoxia-inducible factor 1 (HIF-1): a negative feedback regulatory circuit in HIF-1-mediated signaling in hypoxic cells. *J Biol Chem* 282(19):14073–14082
52. Maynard MA, Evans AJ, Hosomi T, Hara S, Jewett MA, Ohh M (2005) Human HIF-3 α 4 is a dominant-negative regulator of HIF-1 and is down-regulated in renal cell carcinoma. *FASEB J* 19(11):1396–1406
53. Maynard MA, Qi H, Chung J, Lee EH, Kondo Y, Hara S, Conaway RC, Conaway JW, Ohh M (2003) Multiple splice variants of the human HIF-3 α locus are targets of the von Hippel-Lindau E3 ubiquitin ligase complex. *J Biol Chem* 278(13):11032–11040

54. Makino Y, Cao R, Svensson K, Bertilsson G, Asman M, Tanaka H, Cao Y, Berkenstam A, Poellinger L (2001) Inhibitory PAS domain protein is a negative regulator of hypoxia-inducible gene expression. *Nature* 414(6863):550–554
55. Kondo K, Klco J, Nakamura E, Lechpammer M, Kaelin WG (2002) Inhibition of HIF is necessary for tumor suppression by the von Hippel-Lindau protein. *Cancer Cell* 1(3):237–246
56. Raval RR, Lau KW, Tran MG, Sowter HM, Mandriota SJ, Li JL, Pugh CW, Maxwell PH, Harris AL, Ratcliffe PJ (2005) Contrasting properties of hypoxia-inducible factor 1 (HIF-1) and HIF-2 in von Hippel-Lindau-associated renal cell carcinoma. *Mol Cell Biol* 25(13):5675–5686
57. Kondo K, Kim WY, Lechpammer M, Kaelin WG Jr (2003) Inhibition of HIF2 α is sufficient to suppress pVHL-defective tumor growth. *PLoS Biol* 1(3):439–444
58. Zimmer M, Doucette D, Siddiqui N, Iliopoulos O (2004) Inhibition of hypoxia-inducible factor is sufficient for growth suppression of VHL $-/-$ tumors. *Mol Cancer Res* 2(2):89–95
59. Kim WY, Safran M, Buckley MR, Ebert BL, Glickman J, Bosenberg M, Regan M, Kaelin WG Jr (2006) Failure to prolyl hydroxylate hypoxia-inducible factor α phenocopies VHL inactivation in vivo. *EMBO J* 25(19):4650–4662
60. Rankin EB, Rha J, Unger TL, Wu CH, Shutt HP, Johnson RS, Simon MC, Keith B, Haase VH (2008) Hypoxia-inducible factor-2 regulates vascular tumorigenesis in mice. *Oncogene* 27:5354–5358
61. Li L, Zhang L, Zhang X, Yan Q, Minamishima YA, Olumi AF, Mao M, Bartz S, Kaelin WG Jr (2007) Hypoxia-inducible factor linked to differential kidney cancer risk seen with type 2A and type 2B VHL mutations. *Mol Cell Biol* 27(15):5381–5392
62. Purdue MP, Johansson M, Zelenika D, Toro JR, Scelo G, Moore LE, Prokhorchouk E, Wu X, Kiemenev LA, Gaborieau V, Jacobs KB, Chow WH, Zaridze D, Matveev V, Lubinski J, Trubicka J, Szeszenia-Dabrowska N, Lissowska J, Rudnai P, Fabianova E, Bucur A, Bencko V, Foretova L, Janout V, Boffetta P, Colt JS, Davis FG, Schwartz KL, Banks RE, Selby PJ, Harnden P, Berg CD, Hsing AW, Grubb RL, 3rd, Boeing H, Vineis P, Clavel-Chapelon F, Palli D, Tumino R, Krogh V, Panico S, Duell EJ, Quiros JR, Sanchez MJ, Navarro C, Ardanaz E, Dorronsoro M, Khaw KT, Allen NE, Bueno-de-Mesquita HB, Peeters PH, Trichopoulos D, Linseisen J, Ljungberg B, Overvad K, Tjonneland A, Romieu I, Riboli E, Mukeria A, Shangina O, Stevens VL, Thun MJ, Diver WR, Gapstur SM, Pharoah PD, Easton DF, Albanes D, Weinstein SJ, Virtamo J, Vatten L, Hveem K, Njolstad I, Tell GS, Stoltenberg C, Kumar R, Koppova K, Cussenot O, Benhamou S, Oosterwijk E, Vermeulen SH, Aben KK, van der Marel SL, Ye Y, Wood CG, Pu X, Mazur AM, Boulygina ES, Chekanov NN, Foglio M, Lechner D, Gut I, Heath S, Blanche H, Hutchinson A, Thomas G, Wang Z, Yeager M, Fraumeni JF, Jr, Skryabin KG, McKay JD, Rothman N, Chanock SJ, Lathrop M, Brennan P (2011) Genome-wide association study of renal cell carcinoma identifies two susceptibility loci on 2p21 and 11q13.3. *Nat Genet* 43(1): 60–65
63. Gordan JD, Lal P, Dondeti VR, Letrero R, Parekh KN, Oquendo CE, Greenberg RA, Flaherty KT, Rathmell WK, Keith B, Simon MC, Nathanson KL (2008) HIF- α effects on c-Myc distinguish two subtypes of sporadic VHL-deficient clear cell renal carcinoma. *Cancer Cell* 14:435–446
64. Shen C, Beroukhi R, Schumacher SE, Zhou J, Chang M, Signoretti S, Kaelin WG (in press) Genetic and functional studies implicate HIF1 α as a 14q kidney cancer suppressor gene. *Cancer Discov* 1:223–235 (in press)
65. Morris MR, Hughes DJ, Tian YM, Ricketts CJ, Lau KW, Gentle D, Shuib S, Serrano-Fernandez P, Lubinski J, Wiesener MS, Pugh CW, Latif F, Ratcliffe PJ, Maher ER (2009) Mutation analysis of hypoxia-inducible factors HIF1A and HIF2A in renal cell carcinoma. *Anticancer Res* 29(11):4337–4343
66. Lando D, Peet D, Gorman J, Whelan D, Whitelaw M, Bruick R (2002) FIH-1 is an asparaginyl hydroxylase that regulates the transcriptional activity of hypoxia inducible factor. *Genes Dev* 16:1466–1471
67. Hewitson KS, McNeill LA, Riordan MV, Tian YM, Bullock AN, Welford RW, Elkins JM, Oldham NJ, Bhattacharya S, Gleadle JM, Ratcliffe PJ, Pugh CW, Schofield CJ (2002)

- Hypoxia-inducible factor (HIF) asparagine hydroxylase is identical to factor inhibiting HIF (FIH) and is related to the cupin structural family. *J Biol Chem* 277(29):26351–26355
68. Mahon P, Hirota K, Semenza G (2001) FIH-1: a novel protein that interacts with HIF-1 α and VHL to mediate repression of HIF-1 transcriptional activity. *Genes Dev* 15:2675–2686
69. Bracken CP, Fedele AO, Linke S, Balrak W, Lisy K, Whitelaw ML, Peet DJ (2006) Cell-specific regulation of hypoxia-inducible factor (HIF)-1 α and HIF-2 α stabilization and transactivation in a graded oxygen environment. *J Biol Chem* 281(32):22575–22585
70. Yan Q, Bartz S, Mao M, Li L, Kaelin WG Jr (2007) The hypoxia-inducible factor 2{ α } N-terminal and C-terminal transactivation domains cooperate to promote renal tumorigenesis in vivo. *Mol Cell Biol* 27(6):2092–2102
71. Gordan JD, Bertout JA, Hu CJ, Diehl JA, Simon MC (2007) HIF-2 α promotes hypoxic cell proliferation by enhancing c-myc transcriptional activity. *Cancer Cell* 11(4):335–347
72. Gustafsson MV, Zheng X, Pereira T, Gradin K, Jin S, Lundkvist J, Ruas JL, Poellinger L, Lendahl U, Bondesson M (2005) Hypoxia requires notch signaling to maintain the undifferentiated cell state. *Dev Cell* 9(5):617–628
73. Yang H, Minamishima YA, Yan Q, Schlisio S, Ebert BL, Zhang X, Zhang L, Kim WY, Olumi AF, Kaelin WG Jr (2007) pVHL acts as an adaptor to promote the inhibitory phosphorylation of the NF-kappaB agonist Card9 by CK2. *Mol Cell* 28(1):15–27
74. An J, Rettig MB (2005) Mechanism of von Hippel-Lindau protein-mediated suppression of nuclear factor kappa B activity. *Mol Cell Biol* 25(17):7546–7556
75. An J, Fisher M, Rettig MB (2005) VHL expression in renal cell carcinoma sensitizes to bortezomib (PS-341) through an NF-kappaB-dependent mechanism. *Oncogene* 24(9):1563–1570
76. Pantuck AJ, An J, Liu H, Rettig MB (2010) NF-kappaB-dependent plasticity of the epithelial to mesenchymal transition induced by Von Hippel-Lindau inactivation in renal cell carcinomas. *Cancer Res* 70(2):752–761
77. Zhou MI, Wang H, Ross JJ, Kuzmin I, Xu C, Cohen HT (2002) The von Hippel-Lindau (VHL) tumor suppressor stabilizes novel PHD protein Jade-1. *J Biol Chem* 277(42):39887–39898
78. Chitalia VC, Foy RL, Bachschmid MM, Zeng L, Panchenko MV, Zhou MI, Bharti A, Seldin DC, Lecker SH, Dominguez I, Cohen HT (2008) Jade-1 inhibits Wnt signalling by ubiquitylating beta-catenin and mediates Wnt pathway inhibition by pVHL. *Nat Cell Biol* 10(10):1208–1216
79. Peruzzi B, Athauda G, Bottaro DP (2006) The von Hippel-Lindau tumor suppressor gene product represses oncogenic beta-catenin signaling in renal carcinoma cells. *Proc Natl Acad Sci USA* 103(39):14531–14536
80. Mazumdar J, O'Brien WT, Johnson RS, LaManna JC, Chavez JC, Klein PS, Simon MC (2010) O2 regulates stem cells through Wnt/beta-catenin signalling. *Nat Cell Biol* 12(10):1007–1013
81. Semenza GL (2003) Targeting HIF-1 for cancer therapy. *Nat Rev Cancer* 3(10):721–732
82. Welsh S, Williams R, Kirkpatrick L, Paine-Murrieta G, Powis G (2004) Antitumor activity and pharmacodynamic properties of PX-478, an inhibitor of hypoxia-inducible factor-1 α . *Mol Cancer Ther* 3(3):233–244
83. Welsh SJ, Williams RR, Birmingham A, Newman DJ, Kirkpatrick DL, Powis G (2003) The thioredoxin redox inhibitors 1-methylpropyl 2-imidazolyl disulfide and pleurotin inhibit hypoxia-induced factor 1 α and vascular endothelial growth factor formation. *Mol Cancer Ther* 2(3):235–243
84. Mabjeesh NJ, Escuin D, LaVallee TM, Pribluda VS, Swartz GM, Johnson MS, Willard MT, Zhong H, Simons JW, Giannakakou P (2003) 2ME2 inhibits tumor growth and angiogenesis by disrupting microtubules and dysregulating HIF. *Cancer Cell* 3(4):363–375
85. Rapisarda A, Uranchimeg B, Scudiero DA, Selby M, Sausville EA, Shoemaker RH, Melillo G (2002) Identification of small molecule inhibitors of hypoxia-inducible factor 1 transcriptional activation pathway. *Cancer Res* 62(15):4316–4324
86. Rapisarda A, Uranchimeg B, Sordet O, Pommier Y, Shoemaker RH, Melillo G (2004) Topoisomerase I-mediated inhibition of hypoxia-inducible factor 1: mechanism and therapeutic implications. *Cancer Res* 64(4):1475–1482

87. Lee K, Zhang H, Qian DZ, Rey S, Liu JO, Semenza GL (2009) Acriflavine inhibits HIF-1 dimerization, tumor growth, and vascularization. *Proc Natl Acad Sci USA* 106(42):17910–17915
88. Lee K, Qian DZ, Rey S, Wei H, Liu JO, Semenza GL (2009) Anthracycline chemotherapy inhibits HIF-1 transcriptional activity and tumor-induced mobilization of circulating angiogenic cells. *Proc Natl Acad Sci USA* 106(7):2353–2358
89. Zhang H, Qian DZ, Tan YS, Lee K, Gao P, Ren YR, Rey S, Hammers H, Chang D, Pili R, Dang CV, Liu JO, Semenza GL (2008) Digoxin and other cardiac glycosides inhibit HIF-1 α synthesis and block tumor growth. *Proc Natl Acad Sci USA* 105(50):19579–19586
90. Brugarolas JB, Vazquez F, Reddy A, Sellers WR, Kaelin WG Jr (2003) TSC2 regulates VEGF through mTOR-dependent and -independent pathways. *Cancer Cell* 4(2):147–158
91. Arsham AM, Howell JJ, Simon MC (2003) A novel hypoxia-inducible factor-independent hypoxic response regulating mammalian target of rapamycin and its targets. *J Biol Chem* 278(32):29655–29660
92. Treins C, Giorgetti-Peraldi S, Murdaca J, Semenza GL, Van Obberghen E (2002) Insulin stimulates hypoxia-inducible factor 1 through a phosphatidylinositol 3-kinase/target of rapamycin-dependent signaling pathway. *J Biol Chem* 277(31):27975–27981
93. Hudson CC, Liu M, Chiang GG, Otterness DM, Loomis DC, Kaper F, Giaccia AJ, Abraham RT (2002) Regulation of hypoxia-inducible factor 1 α expression and function by the mammalian target of rapamycin. *Mol Cell Biol* 22(20):7004–7014
94. Laughner E, Taghavi P, Chiles K, Mahon PC, Semenza GL (2001) HER2 (neu) signaling increases the rate of hypoxia-inducible factor 1 α (HIF-1 α) synthesis: novel mechanism for HIF-1-mediated vascular endothelial growth factor expression. *Mol Cell Biol* 21(12):3995–4004
95. Zhong H, Chiles K, Feldser D, Laughner E, Hanrahan C, Georgescu MM, Simons JW, Semenza GL (2000) Modulation of hypoxia-inducible factor 1 α expression by the epidermal growth factor/phosphatidylinositol 3-kinase/PTEN/AKT/FRAP pathway in human prostate cancer cells: implications for tumor angiogenesis and therapeutics. *Cancer Res* 60(6):1541–1545
96. Amornphimoltham P, Patel V, Leelahavanichkul K, Abraham RT, Gutkind JS (2008) A ret-roinhibition approach reveals a tumor cell-autonomous response to rapamycin in head and neck cancer. *Cancer Res* 68(4):1144–1153
97. Thomas GV, Tran C, Mellingerhoff IK, Welsbie DS, Chan E, Fueger B, Czernin J, Sawyers CL (2006) Hypoxia-inducible factor determines sensitivity to inhibitors of mTOR in kidney cancer. *Nat Med* 12(1):122–127
98. Guertin DA, Sabatini DM (2007) Defining the role of mTOR in cancer. *Cancer Cell* 12(1):9–22
99. Toschi A, Lee E, Gadir N, Ohh M, Foster DA (2008) Differential dependence of HIF1 α and HIF2 α on mTORC1 and mTORC2. *J Biol Chem* 283:34495–34499
100. Cho DC, Cohen MB, Panka DJ, Collins M, Ghebremichael M, Atkins MB, Signoretti S, Mier JW (2010) The efficacy of the novel dual PI3-kinase/mTOR inhibitor NVP-BEZ235 compared with rapamycin in renal cell carcinoma. *Clin Cancer Res* 16(14):3628–3638
101. Tremblay F, Marette A (2001) Amino acid and insulin signaling via the mTOR/p70 S6 kinase pathway. A negative feedback mechanism leading to insulin resistance in skeletal muscle cells. *J Biol Chem* 276(41):38052–38060
102. Rui L, Fisher TL, Thomas J, White MF (2001) Regulation of insulin/insulin-like growth factor-1 signaling by proteasome-mediated degradation of insulin receptor substrate-2. *J Biol Chem* 276(43):40362–40367
103. O'Reilly KE, Rojo F, She QB, Solit D, Mills GB, Smith D, Lane H, Hofmann F, Hicklin DJ, Ludwig DL, Baselga J, Rosen N (2006) mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. *Cancer Res* 66(3):1500–1508
104. Escudier B, Pluzanska A, Koralewski P, Ravaud A, Bracarda S, Szczylik C, Chevreau C, Filipek M, Melichar B, Bajetta E, Gorbunova V, Bay JO, Bodrogi I, Jagiello-Gruszfeld A, Moore N (2007) Bevacizumab plus interferon alfa-2a for treatment of metastatic renal cell carcinoma: a randomised, double-blind phase III trial. *Lancet* 370(9605):2103–2111

105. Motzer RJ, Hutson TE, Tomczak P, Michaelson MD, Bukowski RM, Rixe O, Oudard S, Negrier S, Szczylik C, Kim ST, Chen I, Bycott PW, Baum CM, Figlin RA (2007) Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. *N Engl J Med* 356(2):115–124
106. Escudier B, Eisen T, Stadler WM, Szczylik C, Oudard S, Siebels M, Negrier S, Chevreau C, Solska E, Desai AA, Rolland F, Demkow T, Hutson TE, Gore M, Freeman S, Schwartz B, Shan M, Simantov R, Bukowski RM (2007) Sorafenib in advanced clear-cell renal-cell carcinoma. *N Engl J Med* 356(2):125–134
107. Sternberg CN, Davis ID, Mardiak J, Szczylik C, Lee E, Wagstaff J, Barrios CH, Salman P, Gladkov OA, Kavina A, Zarba JJ, Chen M, McCann L, Pandite L, Roychowdhury DF, Hawkins RE (2010) Pazopanib in locally advanced or metastatic renal cell carcinoma: results of a randomized phase III trial. *J Clin Oncol* 28(6):1061–1068
108. Hutson TE, Davis ID, Machiels JP, De Souza PL, Rottey S, Hong BF, Epstein RJ, Baker KL, McCann L, Crofts T, Pandite L, Figlin RA (2010) Efficacy and safety of pazopanib in patients with metastatic renal cell carcinoma. *J Clin Oncol* 28(3):475–480
109. Feldman DR, Baum MS, Ginsberg MS, Hassoun H, Flombaum CD, Velasco S, Fischer P, Ronnen E, Ishill N, Patil S, Motzer RJ (2009) Phase I trial of bevacizumab plus escalated doses of sunitinib in patients with metastatic renal cell carcinoma. *J Clin Oncol* 27(9):1432–1439
110. Rini BI, Stein M, Shannon P, Eddy S, Tyler A, Stephenson JJ Jr, Catlett L, Huang B, Healey D, Gordon M (2011) Phase 1 dose-escalation trial of tremelimumab plus sunitinib in patients with metastatic renal cell carcinoma. *Cancer* 117(4):758–767
111. Sosman J, Puzanov I (2009) Combination targeted therapy in advanced renal cell carcinoma. *Cancer* 115(Suppl 10):2368–2375
112. May D, Gilon D, Djonov V, Itin A, Lazarus A, Gordon O, Rosenberger C, Keshet E (2008) Transgenic system for conditional induction and rescue of chronic myocardial hibernation provides insights into genomic programs of hibernation. *Proc Natl Acad Sci USA* 105(1):282–287
113. Schmidinger M, Zielinski CC, Vogl UM, Bojic A, Bojic M, Schukro C, Ruhsam M, Hejna M, Schmidinger H (2008) Cardiac toxicity of sunitinib and sorafenib in patients with metastatic renal cell carcinoma. *J Clin Oncol* 26(32):5204–5212
114. Huang D, Ding Y, Zhou M, Rini BI, Petillo D, Qian CN, Kahnoski R, Futreal PA, Furge KA, Teh BT (2010) Interleukin-8 mediates resistance to antiangiogenic agent sunitinib in renal cell carcinoma. *Cancer Res* 70(3):1063–1071
115. Mizukami Y, Jo WS, Duerr EM, Gala M, Li J, Zhang X, Zimmer MA, Iliopoulos O, Zukerberg LR, Kohgo Y, Lynch MP, Rueda BR, Chung DC (2005) Induction of interleukin-8 preserves the angiogenic response in HIF-1 α -deficient colon cancer cells. *Nat Med* 11(9):992–997
116. Hanrahan EO, Lin HY, Kim ES, Yan S, Du DZ, McKee KS, Tran HT, Lee JJ, Ryan AJ, Langmuir P, Johnson BE, Heymach JV (2010) Distinct patterns of cytokine and angiogenic factor modulation and markers of benefit for vandetanib and/or chemotherapy in patients with non-small-cell lung cancer. *J Clin Oncol* 28(2):193–201
117. Pena C, Lathia C, Shan M, Escudier B, Bukowski RM (2010) Biomarkers predicting outcome in patients with advanced renal cell carcinoma: results from sorafenib phase III treatment approaches in renal cancer global evaluation trial. *Clin Cancer Res* 16(19):4853–4863
118. Choueiri TK, Vaziri SA, Jaeger E, Elson P, Wood L, Bhalla IP, Small EJ, Weinberg V, Sein N, Simko J, Golshayan AR, Sercia L, Zhou M, Waldman FM, Rini BI, Bukowski RM, Ganapathi R (2008) von Hippel-Lindau gene status and response to vascular endothelial growth factor targeted therapy for metastatic clear cell renal cell carcinoma. *J Urol* 180(3):860–865, discussion 865–866
119. Gossage L, Eisen T (2010) Alterations in VHL as potential biomarkers in renal-cell carcinoma. *Nat Rev Clin Oncol* 7(5):277–288
120. Cowey CL, Rathmell WK (2009) VHL gene mutations in renal cell carcinoma: role as a biomarker of disease outcome and drug efficacy. *Curr Oncol Rep* 11(2):94–101
121. Benjamin LE, Golijanin D, Itin A, Pode D, Keshet E (1999) Selective ablation of immature blood vessels in established human tumors follows vascular endothelial growth factor withdrawal. *J Clin Invest* 103(2):159–165

122. Benjamin LE, Hemo I, Keshet E (1998) A plasticity window for blood vessel remodelling is defined by pericyte coverage of the preformed endothelial network and is regulated by PDGF-B and VEGF. *Development* 125(9):1591–1598
123. Bergers G, Song S, Meyer-Morse N, Bergsland E, Hanahan D (2003) Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors. *J Clin Invest* 111(9):1287–1295
124. Rafty LA, Khachigian LM (2002) von Hippel-Lindau tumor suppressor protein represses platelet-derived growth factor B-chain gene expression via the Sp1 binding element in the proximal PDGF-B promoter. *J Cell Biochem* 85(3):490–495
125. Kourembanas S, Hannan RL, Faller DV (1990) Oxygen tension regulates the expression of the platelet-derived growth factor-B chain gene in human endothelial cells. *J Clin Invest* 86:670–674
126. Yoshida D, Kim K, Noha M, Teramoto A (2006) Hypoxia inducible factor 1-alpha regulates of platelet derived growth factor-B in human glioblastoma cells. *J Neurooncol* 76(1):13–21
127. Polite BN, Desai AA, Manchen B, Stadler WM (2006) Combination therapy of imatinib mesylate and interferon-alpha demonstrates minimal activity and significant toxicity in metastatic renal cell carcinoma: results of a single- institution phase II trial. *Clin Genitourin Cancer* 4(4):275–280
128. Vuky J, Isacson C, Fotoohi M, dela Cruz J, Otero H, Picozzi V, Malpass T, Aboulafia D, Jacobs A (2006) Phase II trial of imatinib (Gleevec) in patients with metastatic renal cell carcinoma. *Invest New Drugs* 24(1):85–88
129. Hainsworth JD, Spigel DR, Sosman JA, Burris HA 3rd, Farley C, Cucullu H, Yost K, Hart LL, Sylvester L, Waterhouse DM, Greco FA (2007) Treatment of advanced renal cell carcinoma with the combination bevacizumab/erlotinib/imatinib: a phase I/II trial. *Clin Genitourin Cancer* 5(7):427–432
130. Huang H, Bhat A, Woodnutt G, Lappe R (2010) Targeting the ANGPT-TIE2 pathway in malignancy. *Nat Rev Cancer* 10(8):575–585
131. Yamakawa M, Liu LX, Belanger AJ, Date T, Kuriyama T, Goldberg MA, Cheng SH, Gregory RJ, Jiang C (2004) Expression of angiopoietins in renal epithelial and clear cell carcinoma cells: regulation by hypoxia and participation in angiogenesis. *Am J Physiol Renal Physiol* 287(4):F649–657
132. Currie MJ, Gunningham SP, Turner K, Han C, Scott PA, Robinson BA, Chong W, Harris AL, Fox SB (2002) Expression of the angiopoietins and their receptor Tie2 in human renal clear cell carcinomas; regulation by the von Hippel-Lindau gene and hypoxia. *J Pathol* 198(4):502–510
133. Koochekpour S, Jeffers M, Wang P, Gong C, Taylor G, Roessler L, Stearman R, Vasselli J, Stetler-Stevenson W, Kaelin WJ, Linehan W, Klausner R, Gnarr J, Vande Woude G (1999) The von Hippel-Lindau tumor suppressor gene inhibits hepatocyte growth factor/scatter factor-induced invasion and branching morphogenesis in renal carcinoma cells. *Mol Cell Biol* 19:5902–5912
134. Hara S, Nakashiro KI, Klosek SK, Ishikawa T, Shintani S, Hamakawa H (2006) Hypoxia enhances c-Met/HGF receptor expression and signaling by activating HIF-1alpha in human salivary gland cancer cells. *Oncol* 42(6):593–598
135. Hayashi M, Sakata M, Takeda T, Tahara M, Yamamoto T, Okamoto Y, Minekawa R, Isobe A, Ohmichi M, Tasaka K, Murata Y (2005) Up-regulation of c-met protooncogene product expression through hypoxia-inducible factor-1alpha is involved in trophoblast invasion under low-oxygen tension. *Endocrinology* 146(11):4682–4689
136. Pennacchietti S, Michieli P, Galluzzo M, Mazzone M, Giordano S, Comoglio PM (2003) Hypoxia promotes invasive growth by transcriptional activation of the met protooncogene. *Cancer Cell* 3(4):347–361
137. Bommi-Reddy A, Almeciga I, Sawyer J, Geisen C, Li W, Harlow E, Kaelin WG Jr, Grueneberg DA (2008) Kinase requirements in human cells: III Altered kinase requirements in VHL-/- cancer cells detected in a pilot synthetic lethal screen. *Proc Natl Acad Sci USA* 105(43):16484–16489

138. Lager D, Slagel D, Palechek P (1994) The expression of epidermal growth factor receptor and transforming growth factor alpha in renal cell carcinoma. *Mod Pathol* 7:544–548
139. Petrides P, Bock S, Bovens J, Hofmann R, Jakse G (1990) Modulation of pro-epidermal growth factor, pro-transforming growth factor alpha and epidermal growth factor receptor gene expression in human renal carcinomas. *Cancer Res* 50:3934–3939
140. Ramp U, Jaquet K, Reinecke P, Schardt C, Friebe U, Nitsch T, Marx N, Gabbert HE, Gerharz CD (1997) Functional intactness of stimulatory and inhibitory autocrine loops in human renal carcinoma cell lines of the clear cell type. *J Urol* 157(6):2345–2350
141. Uhlman DL, Nguyen P, Manivel JC, Zhang G, Hagen K, Fraley E, Aeppli D, Niehans GA (1995) Epidermal growth factor receptor and transforming growth factor alpha expression in papillary and nonpapillary renal cell carcinoma: correlation with metastatic behavior and prognosis. *Clin Cancer Res* 1(8):913–920
142. Moch H, Sauter G, Buchholz N, Gasser TC, Bubendorf L, Waldman FM, Mihatsch MJ (1997) Epidermal growth factor receptor expression is associated with rapid tumor cell proliferation in renal cell carcinoma. *Hum Pathol* 28(11):1255–1259
143. Merseburger AS, Hennenlotter J, Simon P, Kruck S, Koch E, Horstmann M, Kuehs U, Kufer R, Stenzl A, Kuczyk MA (2005) Membranous expression and prognostic implications of epidermal growth factor receptor protein in human renal cell cancer. *Anticancer Res* 25(3B):1901–1907
144. Franovic A, Gunaratnam L, Smith K, Robert I, Patten D, Lee S (2007) Translational up-regulation of the EGFR by tumor hypoxia provides a nonmutational explanation for its overexpression in human cancer. *Proc Natl Acad Sci USA* 104(32):13092–13097
145. Humes H, Beals T, Cieslinski D, Sanchez I, Page T (1991) Effects of transforming growth factor-beta, transforming growth factor-alpha, and other growth factors on renal proximal tubule cells. *Lab Invest* 64:538–545
146. Atlas I, Mendelsohn J, Baselga J, Fair W, Masui H, Kumar R (1992) Growth regulation of human renal carcinoma cells: role of transforming growth factor alpha. *Cancer Res* 52(12):3335–9
147. de Paulsen N, Brychzy A, Fournier M-C, Klausner RD, Gnarra JR, Pause A, Lee S (2001) Role of transforming growth factor-alpha in VHL-/- clear cell renal carcinoma cell proliferation: a possible mechanism coupling von Hippel-Lindau tumor suppressor inactivation and tumorigenesis. *Proc Natl Acad Sci USA* 13:1387–1392
148. Smith K, Gunaratnam L, Morley M, Franovic A, Mekhail K, Lee S (2005) Silencing of epidermal growth factor receptor suppresses hypoxia-inducible factor-2-driven VHL-/- renal cancer. *Cancer Res* 65(12):5221–5230
149. Dawson NA, Guo C, Zak R, Dorsey B, Smoot J, Wong J, Hussain A (2004) A phase II trial of gefitinib (Iressa, ZD1839) in stage IV and recurrent renal cell carcinoma. *Clin Cancer Res* 10(23):7812–7819
150. Ravaud A, Hawkins R, Gardner JP, von der Maase H, Zantl N, Harper P, Rolland F, Audhuy B, Machiels JP, Petavy F, Gore M, Schoffski P, El-Hariry I (2008) Lapatinib versus hormone therapy in patients with advanced renal cell carcinoma: a randomized phase III clinical trial. *J Clin Oncol* 26(14):2285–2291
151. Bukowski RM, Kabbinavar FF, Figlin RA, Flaherty K, Srinivas S, Vaishampayan U, Drabkin HA, Dutcher J, Ryba S, Xia Q, Scappaticci FA, McDermott D (2007) Randomized phase II study of erlotinib combined with bevacizumab compared with bevacizumab alone in metastatic renal cell cancer. *J Clin Oncol* 25(29):4536–4541
152. Motzer RJ, Amato R, Todd M, Hwu WJ, Cohen R, Baselga J, Muss H, Cooper M, Yu R, Ginsberg MS, Needle M (2003) Phase II trial of anti-epidermal growth factor receptor antibody C225 in patients with advanced renal cell carcinoma. *Invest New Drugs* 21(1):99–101
153. Rowinsky EK, Schwartz GH, Gollob JA, Thompson JA, Vogelzang NJ, Figlin R, Bukowski R, Haas N, Lockbaum P, Li YP, Arends R, Foon KA, Schwab G, Dutcher J (2004) Safety, pharmacokinetics, and activity of ABX-EGF, a fully human anti-epidermal growth factor

- receptor monoclonal antibody in patients with metastatic renal cell cancer. *J Clin Oncol* 22(15):3003–3015
154. Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, Lindeman N, Gale CM, Zhao X, Christensen J, Kosaka T, Holmes AJ, Rogers AM, Cappuzzo F, Mok T, Lee C, Johnson BE, Cantley LC, Janne PA (2007) MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science* 316(5827):1039–1043
 155. Bean J, Brennan C, Shih JY, Riely G, Viale A, Wang L, Chitale D, Motoi N, Szoke J, Broderick S, Balak M, Chang WC, Yu CJ, Gazdar A, Pass H, Rusch V, Gerald W, Huang SF, Yang PC, Miller V, Ladanyi M, Yang CH, Pao W (2007) MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. *Proc Natl Acad Sci USA* 104(52):20932–20937
 156. Stommel JM, Kimmelman AC, Ying H, Nabioullin R, Ponugoti AH, Wiedemeyer R, Stegh AH, Bradner JE, Ligon KL, Brennan C, Chin L, DePinho RA (2007) Coactivation of receptor tyrosine kinases affects the response of tumor cells to targeted therapies. *Science* 318(5848):287–290
 157. Zhang YW, Staal B, Essenburg C, Su Y, Kang L, West R, Kaufman D, Dekoning T, Eagleson B, Buchanan SG, Vande Woude GF (2010) MET kinase inhibitor SGX523 synergizes with epidermal growth factor receptor inhibitor erlotinib in a hepatocyte growth factor-dependent fashion to suppress carcinoma growth. *Cancer Res* 70(17):6880–6890
 158. Zatyka M, da Silva NF, Clifford SC, Morris MR, Wiesener MS, Eckardt KU, Houlston RS, Richards FM, Latif F, Maher ER (2002) Identification of cyclin D1 and other novel targets for the von Hippel-Lindau tumor suppressor gene by expression array analysis and investigation of cyclin D1 genotype as a modifier in von Hippel-Lindau disease. *Cancer Res* 62(13):3803–3811
 159. Bindra RS, Vasselli JR, Stearman R, Linehan WM, Klausner RD (2002) VHL-mediated hypoxia regulation of cyclin D1 in renal carcinoma cells. *Cancer Res* 62(11):3014–3019
 160. Cairns P, Tokino K, Eby Y, Sidransky D (1995) Localization of tumor suppressor loci on chromosome 9 in primary human renal cell carcinomas. *Cancer Res* 55(2):224–227
 161. Costes V, Liautaud J, Picot MC, Robert M, Lequeux N, Brochier J, Baldet P, Rossi JF (1997) Expression of the interleukin 6 receptor in primary renal cell carcinoma. *J Clin Pathol* 50(10):835–840
 162. Takenawa J, Kaneko Y, Fukumoto M, Fukatsu A, Hirano T, Fukuyama H, Nakayama H, Fujita J, Yoshida O (1991) Enhanced expression of interleukin-6 in primary human renal cell carcinomas. *J Natl Cancer Inst* 83(22):1668–1672
 163. Miki S, Iwano M, Miki Y, Yamamoto M, Tang B, Yokokawa K, Sonoda T, Hirano T, Kishimoto T (1989) Interleukin-6 (IL-6) functions as an in vitro autocrine growth factor in renal cell carcinomas. *FEBS Lett* 250(2):607–610
 164. van Rossum AP, Vlasveld LT, Vlasveld IN, Jansen PM, Dik WA, Hooijkaas H, Castel A (2009) Granulocytosis and thrombocytosis in renal cell carcinoma: a pro-inflammatory cytokine response originating in the tumour. *Neth J Med* 67(5):191–194
 165. Perut F, Cenni E, Unger RE, Kirkpatrick CJ, Giunti A, Baldini N (2009) Immunogenic properties of renal cell carcinoma and the pathogenesis of osteolytic bone metastases. *Int J Oncol* 34(5):1387–1393
 166. Horiguchi A, Oya M, Marumo K, Murai M (2002) STAT3, but not ERKs, mediates the IL-6-induced proliferation of renal cancer cells, ACHN and 769P. *Kidney Int* 61(3):926–938
 167. Rossi JF, Negrier S, James ND, Kocak I, Hawkins R, Davis H, Prabhakar U, Qin X, Mulders P, Berns B (2010) A phase I/II study of siltuximab (CNTO 328), an anti-interleukin-6 monoclonal antibody, in metastatic renal cell cancer. *Br J Cancer* 103(8):1154–1162
 168. Chiche J, Fur YL, Vilmen C, Frassinetti F, Daniel L, Halestrap AP, Cozzzone PJ, Pouyssegur J, Lutz NW (2011) In vivo pH in metabolic-defective Ras-transformed fibroblast tumors: key role of the monocarboxylate transporter, MCT4, for inducing an alkaline intracellular pH. *Cancer epub ahead of print*
 169. Ullah MS, Davies AJ, Halestrap AP (2006) The plasma membrane lactate transporter MCT4, but not MCT1, is up-regulated by hypoxia through a HIF-1alpha-dependent mechanism. *J Biol Chem* 281(14):9030–9037

170. Perez de Heredia F, Wood IS, Trayhurn P (2010) Hypoxia stimulates lactate release and modulates monocarboxylate transporter (MCT1, MCT2, and MCT4) expression in human adipocytes. *Pflugers Arch* 459(3):509–518
171. Le A, Cooper CR, Gouw AM, Dinavahi R, Maitra A, Deck LM, Royer RE, Vander Jagt DL, Semenza GL, Dang CV (2010) Inhibition of lactate dehydrogenase A induces oxidative stress and inhibits tumor progression. *Proc Natl Acad Sci USA* 107(5):2037–2042
172. Xie H, Valera VA, Merino MJ, Amato AM, Signoretti S, Linehan WM, Sukhatme VP, Seth P (2009) LDH-A inhibition, a therapeutic strategy for treatment of hereditary leiomyomatosis and renal cell cancer. *Mol Cancer Ther* 8(3):626–635
173. Schneiderhan W, Scheler M, Holzmann KH, Marx M, Gschwend JE, Bucholz M, Gress TM, Seufferlein T, Adler G, Oswald F (2009) CD147 silencing inhibits lactate transport and reduces malignant potential of pancreatic cancer cells in in vivo and in vitro models. *Gut* 58(10):1391–1398
174. Fantin VR, St-Pierre J, Leder P (2006) Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology, and tumor maintenance. *Cancer Cell* 9(6):425–434

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Figlin, R.A.; Rathmell, W.K.; Bloom, J. (Eds.)

2012, XII, 328 p., Hardcover

ISBN: 978-1-4614-2399-7